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PRINCIPAL INVESTIGATOR: **George Tsokos, M.D.**

CONTRACTING ORGANIZATION: **Beth Israel Deaconess Medical Center
Boston, MA**

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14. ABSTRACT Our goal is to ultimately enable the development of novel therapeutic agents and strategies to ameliorate tissue injury in I/R related conditions. Preliminary findings suggested a role for IL-17 & IL-23 axis in ischemia reperfusion injury which we have continued to study. We have shown that by depleting IL-17 and IL-23 tissue injury is alleviated. Our findings unravel the therapeutic role of R-spondin 3 in protecting tissues from damage by preventing endothelial barrier dysfunction and dampening I/R enhanced local inflammatory response. We are now exploring in depth the regulatory mechanisms by which R-spo3 plays its protective role in tissue injury for therapeutic purposes.					
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Introduction

Tissue injury due to mesenteric ischemia/reperfusion is a common pathology that is frequently encountered in a variety of clinical and surgical conditions including shock, trauma, small bowel transplantation, sepsis, and cardiovascular interventions. Ischemia induces local cellular changes such as membrane perturbation, cytoskeletal disorganization, upregulation of adhesion molecules, neo-antigen expression and increased permeability. These alterations provoke endothelial dysfunction, intense inflammatory cascade, increased mucosal permeability and tissue necrosis during subsequent reperfusion (1-10). The susceptibility of the ischemic tissue to the ensuing catastrophic events caused during the re-establishment of blood, leads to a phenomenon known as ischemia/reperfusion (I/R) injury. The restored blood flow also causes systemic inflammation affecting remote organs resulting in systemic inflammatory response syndrome and multiple organ dysfunction syndromes that are associated with high rates of morbidity and mortality (10).

Several molecules and cells have been implicated in I/R injury, such as reactive oxygen species (ROSs), cytokines and chemokines (11), adhesion molecules (12,13), complement (14,15), natural antibodies (16-18), B cells (19), T cells (20, 21), neutrophils (22), endothelial cells and platelets (23). Deposition of natural antibodies, subsequent complement activation and neutrophil infiltration represent the initiating events in this process (9). Recently, the role of different T cell subsets (Tregs, Th17) and B cells in I/R injury has been considered as well (24, 25). In addition, there are increasing reports on the role of gut microbiota in acute intestinal inflammation and injury (26). Data from the ongoing studies clearly show that I/R injury is a very complex process that literally recruits all the components of the immune response. To date there is no specific therapy that has yet gained widespread clinical use for the prevention and treatment of ischemic related conditions.

Others and we have shown that natural antibodies are central in the pathogenesis of mesenteric I/R injury and initiate I/R-induced tissue injury (14, 16, 17). The observations led to the identification of neo-antigens such as annexin IV and β -actin on the surface of the ischemic cells

to which natural IgM bind and subsequently activate complement (27, 28). Deficiency of complement factors has been shown to be protective against I/R injury (14), and inhibition of the complement pathway through various interventions have been reported to attenuate I/R-induced damage (29). These findings clearly indicate the proinflammatory behavior of these key molecules in I/R injury and their potential to be targets for therapeutic intervention. We are continuing our efforts to further understand and reveal the underlying mechanisms associated with different aspects of I/R pathology and counter the processes using various pharmacological agents and therapeutic strategies.

Body

(1) Complement 3 in ischemia injury

Over the years exclusive emphasis has been placed on the role of the reperfusion, which introduces antibodies and complement to the ischemia-conditioned tissue and exacerbates the tissue damage. However, it is important to note that ischemia sets the stage for reperfusion injury and therefore, understanding the mechanisms involved in the development of tissue damage at this early phase are essential in improving the clinical outcome of life-threatening conditions such as trauma/hemorrhagic shock, organ transplantation, cardiovascular and autoimmune diseases. The complement system is an important mediator of IR injury in various organs including intestine, kidney, liver, lungs and heart. In mesenteric IR complement activation and deposition have been recognized as key components for the initiation and amplification of the inflammatory response which occurs during reperfusion injury. Numerous studies have demonstrated the value of complement inhibition in the mitigation of reperfusion-induced tissue injury including complement depletion with cobra venom factor (CVF) and blockade of the classical, alternative and lectin pathways.

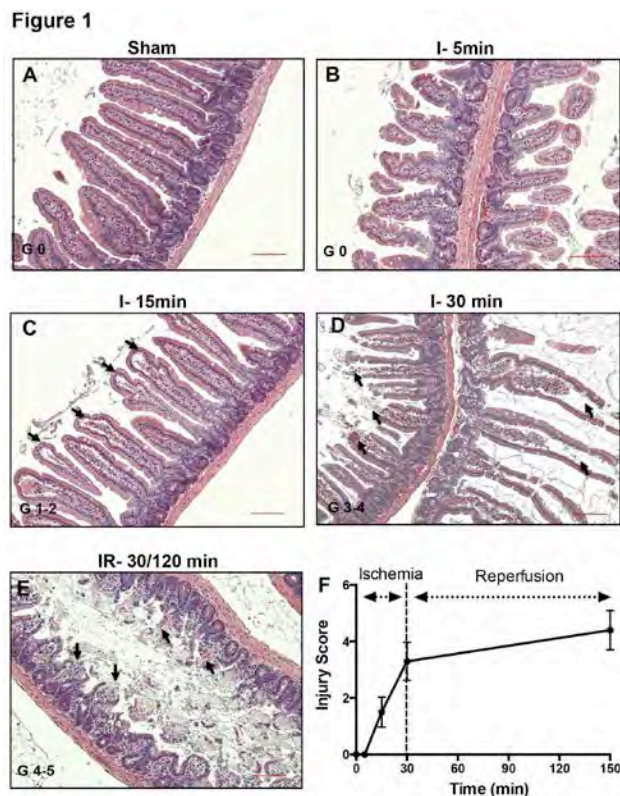
It was recently recognized that besides the well-established pathways of complement activation complement can be activated within the cytoplasm prior to its secretion. Furthermore, complement is produced in extrahepatic sites and this local production appears to be involved in the inflammatory response. For example, C4 produced by monocytes restores humoral immunity in C4-deficient mice and C3 production by kidney epithelial cells regulates acute renal transplant rejection. Recently, human CD4⁺ T cells were found to produce C3 which was cleaved intracellularly by the protease cathepsin L suggesting a role for C3 in T cell homeostasis.

In this study, we have revisited carefully the histological events that occur during mesenteric ischemia and reperfusion in the intestine. We were surprised to observe significant damage in the intestinal villi during the first 30 minutes following closure of the superior mesenteric artery suggesting that injury occurs at an early phase prior to reperfusion and that reperfusion solely amplifies the inflammatory damage. We present evidence that locally produced C3 contributes to ischemic injury and its primary source is the villus epithelium. We further show that Caco2

intestinal epithelial cells produce C3 along with other complement components and that intracellular cathepsin B cleaves C3 to produce C3a. Thus, we propose that locally produced complement components activated by intracellular proteases are responsible for the damage that we observed during the ischemia phase.

Mesenteric ischemia instigates and reperfusion amplifies tissue damage in small intestine.

First we asked whether and to what degree mesenteric ischemia contributes to mucosal damage of the intestine. Figure 1 depicts representative images of intestinal tissue from mice subjected to continuous mesenteric ischemia for 5, 15 and 30 minutes without reperfusion (Fig 1B-D), continuous mesenteric ischemia for 30 min followed by 120 min reperfusion (Fig 1E) or sham operation (Fig 1A). Using Chiu-scoring analysis on the distribution of histological injury grades, we observed onset of damage after 15 min of ischemia with lesions of grades 1 and 2, which represent the development of a subepithelial space near the tips and the extension of the subepithelial space on both sides of the villi (Fig. 1C). By 30 min of ischemia the damage progressed to grades 3 and 4 (Fig. 1D), which represent massive epithelial uplifting, denudation of the tips and increased cellularity of the lamina propria. The degree of epithelial damage in the villi significantly increased with prolonged ischemia with the majority of villi falling into grade 5 in mice subjected to 45- and 60-min ischemia (data not shown), marked by denudation of the villi, disintegration of the lamina propria and hemorrhage. As expected, ischemia-reperfusion of

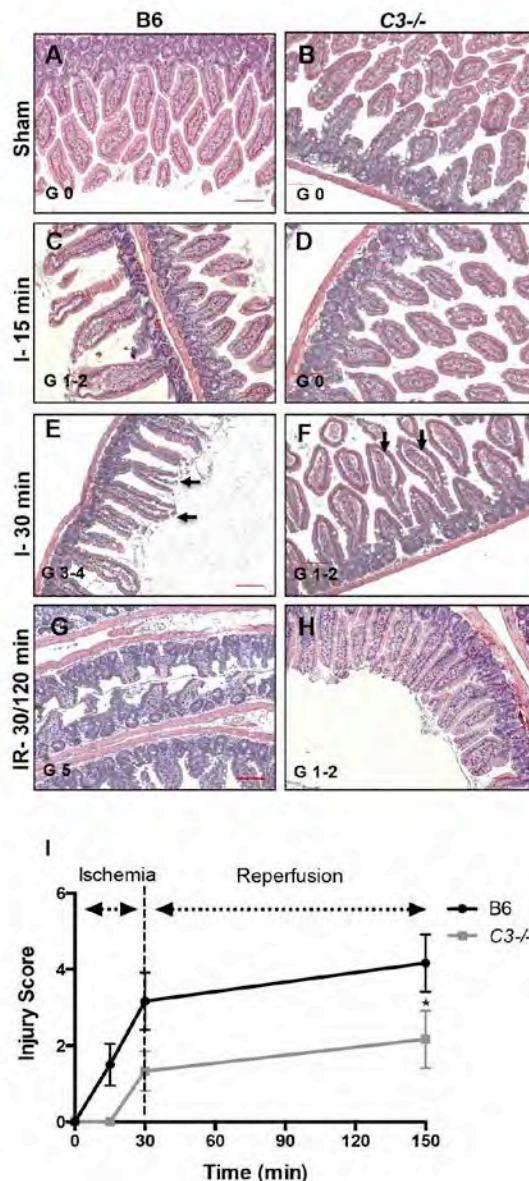


30/120 min resulted in severe epithelial damage with grades 4 and 5 and a small component of grade 3. Cumulative data of intestinal injury evaluated at different conditions is shown in Fig. 1F. Thus, these results demonstrate that mesenteric ischemia can alone initiate epithelial damage which is subsequently amplified if the intestine is reperused.

C3 deficiency limits intestinal damage during mesenteric ischemia

We considered that complement may play a role in the expression damage which occurs during ischemia. Accordingly, we subjected C3-deficient mice to different periods of ischemia or ischemia followed by reperfusion. C3-sufficient or C3-deficient animals subjected to sham procedures did not display any damage of the villi (Fig. 2A and B). Damage of the villi was noted in normal mice subjected to 15 (Fig. 2C) or 30 min of ischemia (Fig. 2E) or 30 min of

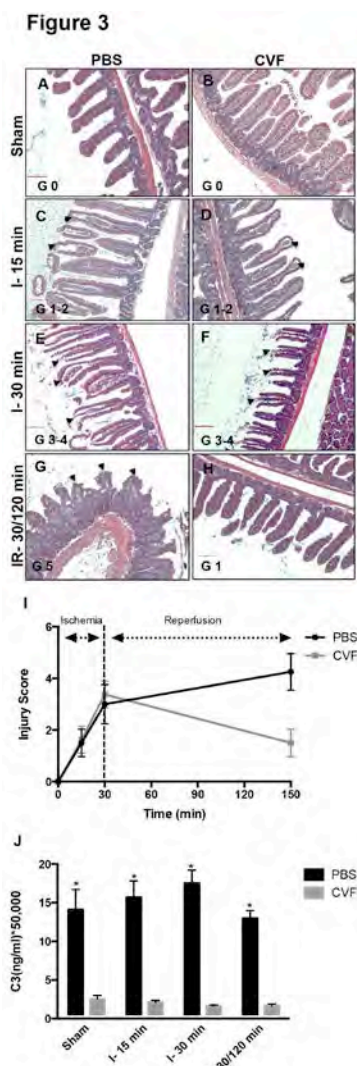
Figure 2



ischemia followed by 120 min of reperfusion (Fig. 2G). Unlike their sufficient counterparts, C3-deficient mice displayed limited intestinal damage during ischemia and as expected (16) after reperfusion. Specifically, the integrity of the intestinal villi in the C3-deficient mice subjected to 15 min of ischemia remained intact (Fig. 2D) and was only minimally compromised (grade 1 lesions) after 30 min of ischemia (Fig. 2F). Consistent with previously published studies (16), C3-deficient mice subjected to 30/120 min IR (Fig. 2H) showed a remarkable decrease in the epithelial damage when compared with normal mice. Fig. 2I presents cumulative data and demonstrates a significant reduction in the intestinal injury score in C3-deficient compared to C3-sufficient mice. Together, these data suggest that C3 contributes to the development of tissue damage during ischemia.

Complement depletion by cobra venom factor (CVF) prevents ischemia-reperfusion-induced injury but not ischemia-induced injury

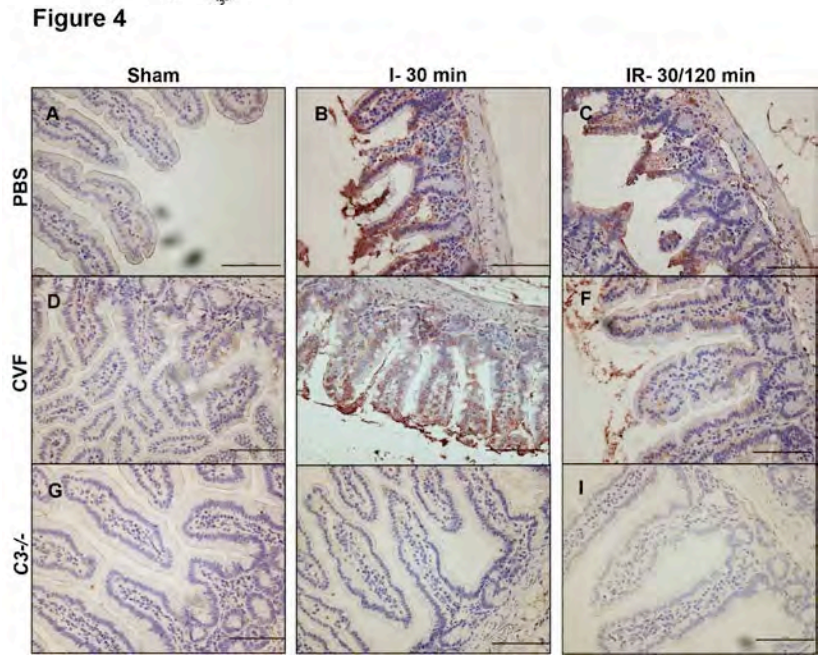
Complement depletion by CVF has been demonstrated repeatedly to protect mice from tissue damage in various IR models (17-20). CVF forms a stable convertase with factor B that is



resistant to hydrolysis and continuously activates C3 through the alternative pathway, resulting in depletion of complement in the circulation. To address whether circulating complement is also responsible for the intestinal lesions that we observed during ischemia, we administered CVF (12 U) to mice 24 and 16 hours prior to mesenteric ischemia or IR or sham procedures. As expected, mice treated with CVF had negligible levels of C3 in their serum at the time of euthanasia (determined by ELISA) compared to PBS administered animals (Fig. 3J). In agreement with previous studies, we observed limited (grade 1 and 2) IR-induced lesions in CVF-treated (Fig. 3G) as opposed to grade 4 and 5 lesions in PBS-treated (Fig. 3H) mice. Interestingly however, the degree of lesions in animals subjected to 15 and 30 min of ischemia was comparable among CVF- and PBS-treated mice (Fig. 3C-F). Cumulative data of intestinal injury evaluated at different conditions is shown in Fig. 3I. Collectively, these findings demonstrate that although circulating C3 is an important mediator of the injury that follows reperfusion, it is not required for the injury, which occurs during ischemia.

Intracellular production and activation of C3 in intestinal epithelial cells

Several reports have provided evidence that acute inflammatory conditions including sepsis and endotoxemia can stimulate the production of C3 in intestinal



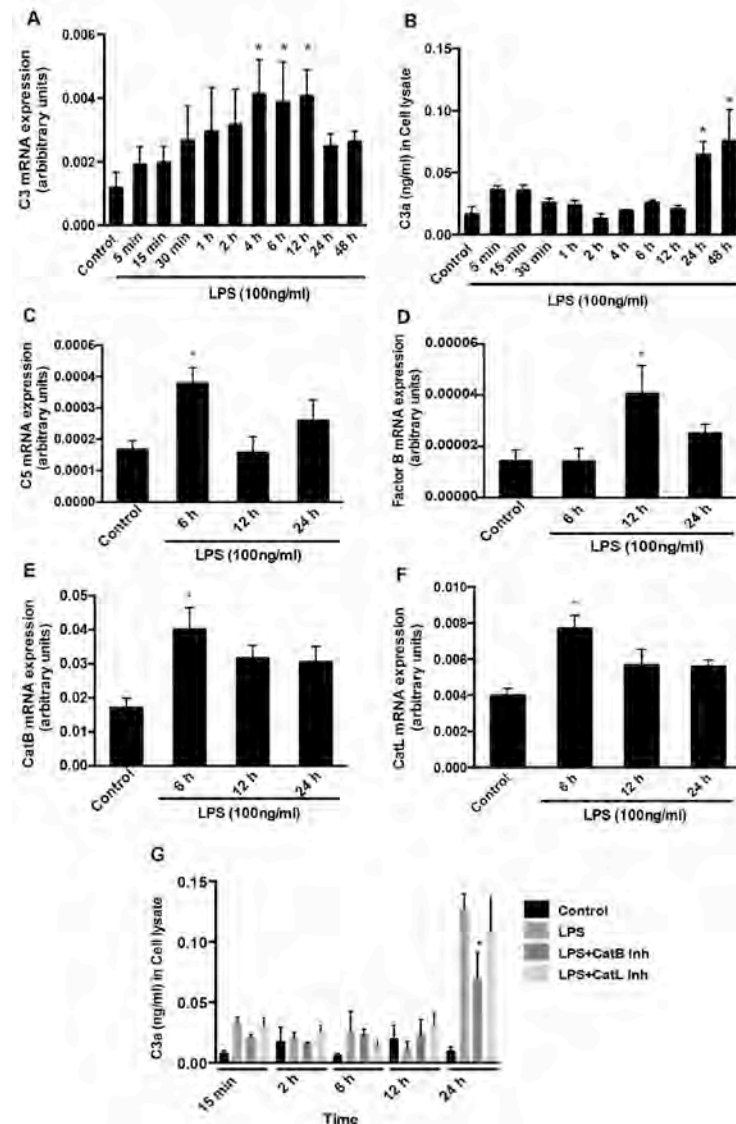
mucosa and that the enterocyte is a major source of C3 (21). Having shown that circulating complement is not the cause of the ischemia-associated intestinal injury, we addressed whether locally produced complement may be a contributing factor. Therefore, we evaluated intestinal tissue sections obtained from normal, CVF-treated and C3-deficient mice by immunohistochemistry using an anti-C3 antibody that detects complement precursor C3 and fragments of C3 including c3a, c3b and c3c. Intestinal tissue sections from sham-operated mice displayed weak staining (Fig. 4A), whereas sections obtained from mice subjected to 30 min of ischemia or 30/120 IR showed a strong signal (Fig. 4B and C). Staining for C3/C3 fragments was predominant in the injured villi and primarily along the terminally differentiated epithelium and in few cells in the lamina propria. Interestingly, positive staining for C3/C3 fragments was also noted in the sections of mice treated with CVF and subjected to 30 min of ischemia and this staining was comparable to that recorded in mice treated with PBS (Fig. 4B and E). However, the reperfusion-enhanced C3 expression in intestinal tissues was reduced in CVF-treated mice (Fig. 4F). Sections from C3-deficient mice subjected to 30 min of ischemia did not reveal any staining (Fig. 4G-I). Our finding of strong expression of C3/C3 fragments in injured intestinal epithelium during ischemia, even in the absence of circulating complement (treatment with CVF) favors the involvement of locally produced complement in the ischemic phase of intestinal injury.

To show the potential of intestinal epithelial cells to produce and activate complement, we used an *in vitro* culture of human colonic transformed cells Caco2 and incubated them at various time intervals with LPS (100ng/ml), a potent inducer of C3 production. We used Caco-2 cells because they differentiate spontaneously forming polarized monolayers with tight junctions and brush border microvilli at apical surfaces and thus simulate the enterocytes of small intestine (22). As shown in Fig. 5A, unstimulated Caco2 cells expressed low levels of C3 and the addition of LPS induced a time-dependent increase in the C3 mRNA levels. C3 mRNA expression increased as early as 5 min and continued to gradually increase and became statistically significant at 4, 6 and 12 hours. We then evaluated C3 activation by measuring the levels of C3a in cell lysates by ELISA. C3a is a small cleavage fragment released by C3 activation and is a potent mediator of inflammation. Interestingly, we observed a spike in C3a levels between 5 and 30 min in the cell lysate that increased significantly at 24 and 48 hours as depicted in Fig. 5B. In addition, we

measured the mRNA levels of other complement factors such as C5 and factor B. Both were found increased at 6 hours after LPS stimulation (Fig. 5C and D).

Having seen that Caco2 cells produce C3a we considered that C3 is activated intracellularly by

Figure 5



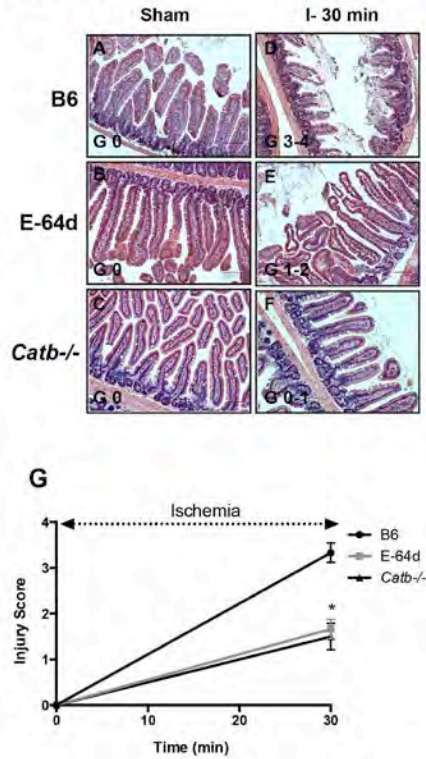
proteases. We searched for cathepsin expression in Caco2 cells by qPCR and found that unlike T cells, which express only Cathepsin L (CatL), Caco2 cells express Cathepsin B (CatB) and CatL. Gene expression analysis revealed the presence of large amounts of mRNA coding for CatB and CatL in resting Caco2 cells, which increased upon LPS activation after 6 hours (Fig. 5E and F), whereas CatG was absent (data not shown). Based on evidence that these proteases mediate intracellular C3 activation (14), we tested the cleavage of C3 in Caco2 cells in the presence of CatB and CatL inhibitors. We incubated Caco2 cells with CatB and CatL-specific inhibitors an hour prior to LPS stimulation, harvested cell lysates at different time points and measured C3a levels by ELISA. At 24 hours

cells treated with LPS and the CatB inhibitor, but not the CatL inhibitor, showed a statistically significant decrease in the levels of C3a compared with cells treated only with LPS (Fig. 5G). These *in vitro* studies reveal for the first time that the cysteine protease-CatB can cleave intracellular C3 in the Caco2 intestinal epithelial cells.

Cathepsin inhibition or genetic deficiency limits intestinal injury caused by ischemia

To determine whether cathepsin B is responsible for C3 cleavage and intestinal damage during ischemia, we treated mice with a cathepsin inhibitor prior to

Figure 6



ischemia, we treated mice with a cathepsin inhibitor prior to subjecting them to ischemia or ischemia and reperfusion. For this study, we chose to use the cathepsin inhibitor E-64d, which has been reported to block the activation of cathepsins including B, L, and H. As illustrated in Fig. 6A and B, intestinal tissue integrity was maintained in sham-operated animals in both groups. Mice that received PBS and were subjected to mesenteric ischemia for 30-min without reperfusion showed damage of grade 3-4 (Fig. 6D) which was decreased significantly after treatment with E-64d administered 30 min prior to the initiation of ischemia (Fig. 6E). Control mice that received PBS and subjected to 30-min ischemia followed by 120 reperfusion exhibited severe grade 5 lesions (data not shown).

Next, we subjected CatB-deficient mice (*Catb*^{-/-}) to ischemia and noted that these mice, unlike the control did not experience any injury during the first 30 min of ischemia (Fig. 6F). The sham-operated *Catb*^{-/-} mice showed no damage (Fig. 6C). Taken together, these data suggest that cathepsin B is involved in the intracellular activation of C3 and its inhibition attenuates injury induced during mesenteric ischemia. Cumulative data from these experiments are shown in Fig. 6G.

(2) IL-17 in ischemia-reperfusion injury

IL-17A is a cytokine that causes epithelial cells to secrete neutrophil chemoattractant chemokines such as CXCL2 and IL-8. Cells that produce IL-17A include TCR α/β T cells, TCR γ/δ T cells and CD45⁺CD4⁺TCR-IL-7R⁺ innate lymphoid cells (ILCs), which reside at mucosal surfaces. Other sources of IL-17A include dendritic cells (DCs), macrophages, neutrophils, natural killer cells (NK) and mast cells. The IL17A receptor (IL-17AR) is a heterodimer of the IL-17RA and IL-17RC chains that binds IL-17A and its homologue IL-17F

and is expressed predominantly on epithelial cells. Because of its ability to mobilize neutrophils, IL-17A is important in the pathogenesis of autoimmune diseases, and in chronic steroid resistant asthma characterized by neutrophil predominance. It is also important for host defense against candida infection. This is illustrated by the susceptibility of patients with mutations in IL-17A and its receptor to candida infections.

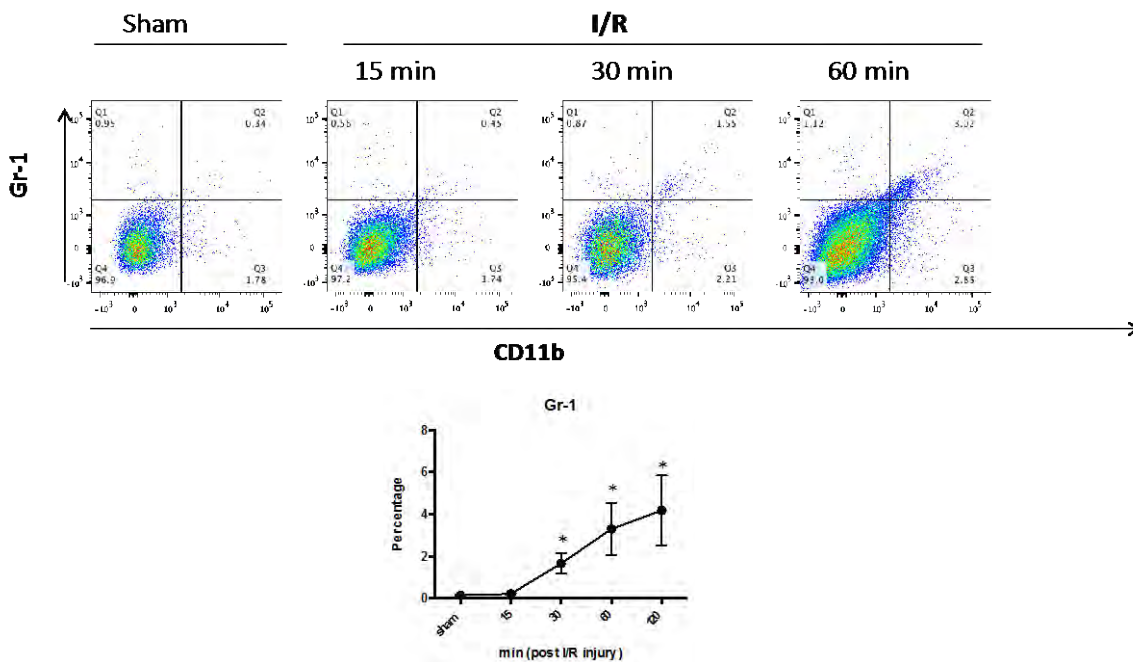
Naïve TCR α/β cells differentiate into T helper 17 (Th17) cells following TCR ligation in the presence of the inflammatory cytokines IL-1, IL-6 and TGF β . Th17 differentiation is promoted by the cytokine IL-23. In contrast, IL-17A production by TCR γ/δ T cells ILCs and other innate cell is directly driven by IL-23 and is rapid. IL-23 is a heterodimer of the 40 kD chain (p40) shared with IL-12 and the IL-23 specific 19 kD chain p19, and is produced by epithelial cells and DCs. The IL-23R is a heterodimer of the IL12R β 1 chain, shared with the IL-12R, and an IL-23R specific chain, which associates constitutively with Janus kinase 2 and binds to the transcription activator STAT3 in a ligand-dependent manner.

It was previously shown that tissue-infiltrating CD4⁺ T cells in IRI produce IL-17A. It has been recently suggested that IL-17A is important for small intestinal IRI, and that Paneth cells store IL-17A and are the major source of IL-17A in IRI. However, since T cells are not essential for IRI, and the published gene array analyses do not show that Paneth cells express Il17a, we set out to analyze the source of IL-17A relevant to IRI. We have confirmed the role of IL-17A in IRI and demonstrated a role for IL-23. Our analysis of the sources of these two cytokines in IRI is ongoing.

Increased neutrophil infiltration in the lamina propria (LP) in IRI.

Local infiltration with neutrophils has been well documented in IRI, based on histologic examination of intestinal tissue sections and considered responsible for the development of injury. Because the intestinal lesions in IRI can be patchy, and in order to measure neutrophil infiltration quantitatively, we determined the total number of neutrophils in the LP of intestine of mice subjected to IR. Cell suspensions were prepared from the LP of the entire small intestine and analyzed by flow cytometry analysis for CD45 expression. The percentage of neutrophils among the gated CD45⁺ cells was determined using CD11b and Gr1 as markers. IRI resulted in an increase in the percentage and number of CD45⁺ cells in the LP and in a time-dependent increase in the percentage of neutrophils in the CD45⁺ fraction of cells (Fig. 1). These results

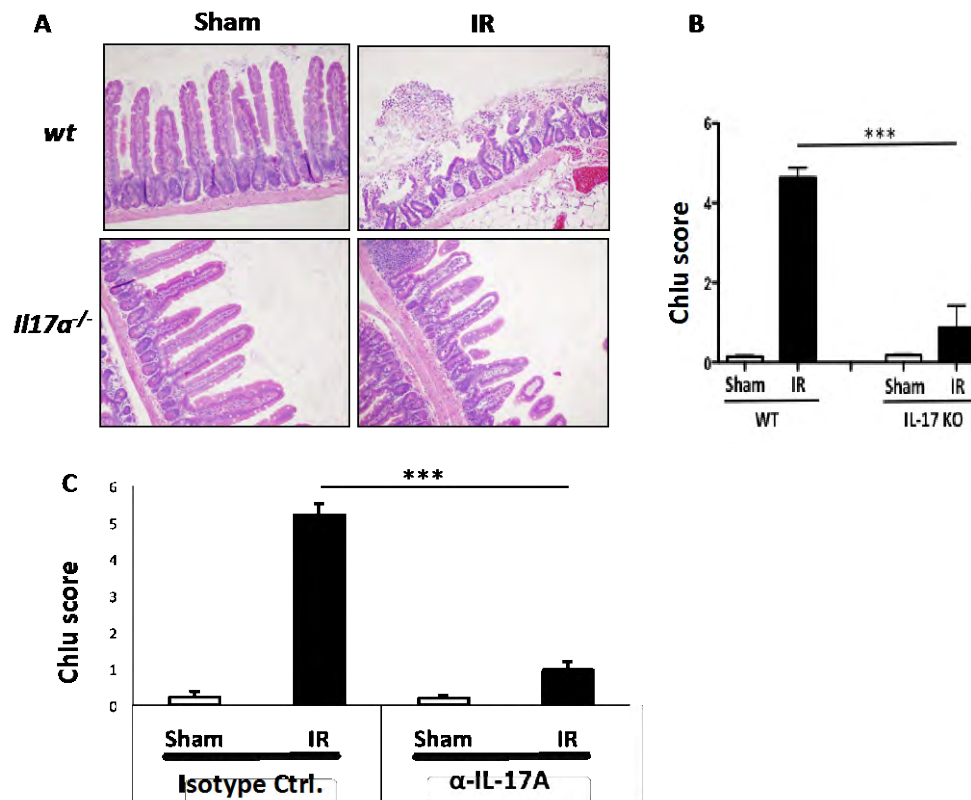
demonstrate that IRI causes a time dependent accumulation of neutrophils in the LP of the small intestine.



IL-17A is important for IRI. IL-17A was reported to be important for IRI in mice on the C57BL/6 background (31). To confirm the role of IL-17A in IRI in the BALB/c background, we subjected *Il17a*^{-/-} BALB/c mice and their genetically matched WT controls to IRI. WT BALB/c mice developed severe intestinal damage following IR as evidenced by loss of villi, neutrophilic infiltration of the LP and fibrin deposition (Fig. 2 A,B) and an injury score of 4.7 compared to 0.4 in sham-operated BALB/c WT controls. In contrast, *Il17a*^{-/-} mice developed markedly less intestinal damage compared to WT controls, with preservation of villous architecture lack of cellular infiltration. The injury score was 0.87 compared to 0.3 in sham-operated *Il-17a*^{-/-} control mice.

Lack of IL17-A could avert IRI injury because of developmental or unknown environmental factors. To confirm the importance of IL-17A in IRI, WT mice were treated with a neutralizing IgG antibody to IL-17A, or IgG isotype control antibody, on days -4, -2 and -1 prior to IR. WT mice treated with neutralizing IL-17A antibody developed significantly less small intestinal (SI) damage after IR than mice treated with isotype control antibody as demonstrated by significantly lower injury scores compared to IgG isotype-treated control mice (Fig. 2C). These results

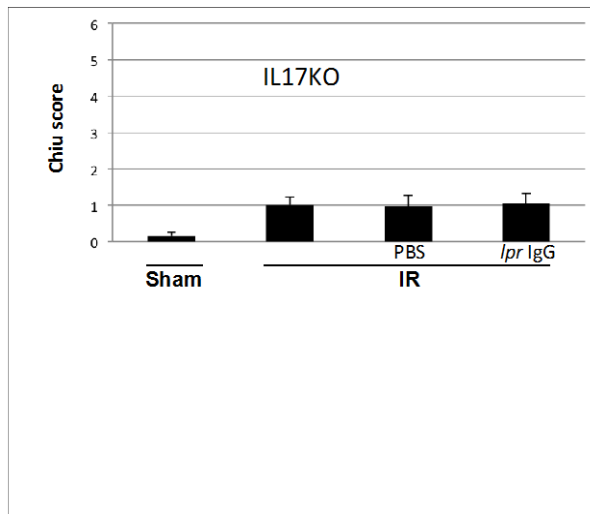
demonstrate that IL-17A is important for IRI regardless of genetic background and independent of any potential derangements due to IL-17A deficiency.



IL-17A is downstream from the early events of small intestinal IRI.

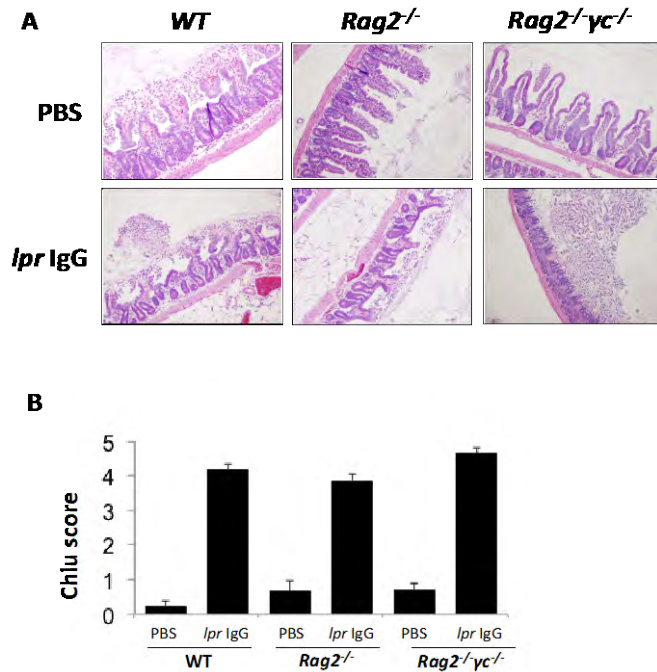
Ischemic injury induces the expression by damaged cells of antigens that are recognized by circulating natural antibodies, which fix and activate complement (36). This is supported by the observation that IgM, IgG and C3 are deposited in the small intestine during IRI, by the abrogation of IRI in the absence of complement and the ability of serum from WT and lupus-prone MRL.*lpr* mice to restore IRI in *Rag*-deficient IRI-resistant mice. We asked whether IL-17 precedes or follows the deposition of Ig and complement action in the expression of IRI injury. In considering that *Il17a^{-/-}* mice could lack natural antibodies in a manner similar to that observed in *Cr2*-deficient mice, we administered purified IgG from MRL.*lpr* mice 30 min prior to the initiation of IR. Injection of lupus mouse derived Ig into *Il17a^{-/-}* mice failed to restore IRI (Fig. 3A). The same batch of IgG restored IRI in *Rag2^{-/-}* mice in a manner similar to that shown

before (data not shown). These results suggest that IL-7A is downstream of antibody- and complement-mediated triggering of IRI.



ILCs are not the source of IL-17A in small intestinal IRI.

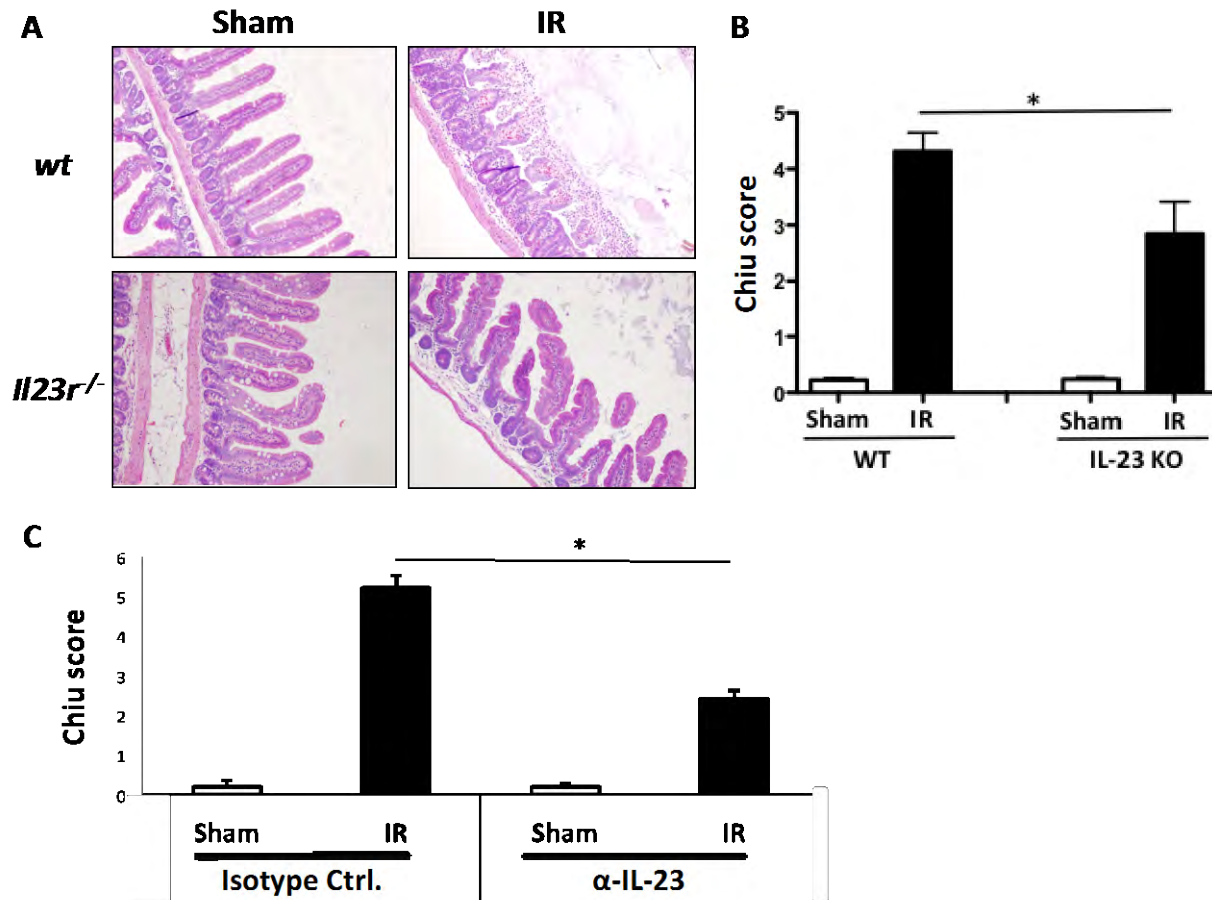
It has been recently suggested that IL-17A is important for small intestinal IRI, and that Paneth cells store IL-17A and are the major source of IL-17A in IRI (31). However the published gene expression analyses do not show that Paneth cells express *Il17a* mRNA. Since T cells may be dispensable for IRI, as IRI develops in Rag deficient mice reconstituted with serum from MRL.*lpr* mice. This prompted us to analyze the potential cellular source of IL-17A important for small intestinal IRI. We first tested the hypothesis that ILCs, which rapidly release IL-17A in response to epithelial injury, are the relevant source of IL-17A in small intestinal IRI. To address this question we took advantage of the *Rag2*^{-/-} γ ^{-/-} mouse, which lacks ILCs because it is deficient in IL-7 signaling, which requires the γ c containing IL-7R. Since *Rag2*^{-/-} γ ^{-/-} mice, like *Rag2*^{-/-} mice, lack immunoglobulins, we administered IgG from MRL.*lpr* mice to *Rag2*^{-/-} γ ^{-/-} mice prior to subjecting them to IR. Infusion of lupus-derived Ig reconstituted IRI in *Rag2*^{-/-} γ ^{-/-} mice even better than it did in *Rag2*^{-/-} mice (Fig 4. A,B). These results indicate that ILCs are not the relevant source of IL-17A in small intestinal IRI and suggest that ILCs may in fact protect against small intestinal IRI.



Cellular source of IL-17A in small intestinal IRI. In view of the above negative result and to ascertain whether a hematopoietic cell is the source of IL-17A in small intestinal IRI, we constructed bone marrow (BM) radiation chimeras. To determine the contribution of IL-17A derived from hematopoietic cells in small intestinal IRI, we generated chimeras in which BM from CD45.2⁺ WT and *Il17a*^{-/-} donors was used to reconstitute sublethally irradiated CD45.1⁺ WT recipients. To determine the contribution of IL-17A derived from non-hematopoietic cells BM from CD45.1⁺ WT donors was used to reconstitute sublethally irradiated CD45.2⁺ WT or *Il17a*^{-/-} recipients. FACS analysis revealed that after 8 weeks >90% of the blood cells in the BM chimeras were donor-derived. Nine weeks after BM reconstitution, the chimeras were subjected to IRI. Both WT->WT chimeras and WT->*Il17a*^{-/-} chimeras underwent IRI comparable to WT controls. *Il17a*^{-/-} ->WT chimeras had markedly attenuated IRI (5B). These results indicate that the cellular source of IL-17A required for intestinal damage after intestinal IRI is of hematopoietic origin.

IL-23 is important for small intestinal IRI. The cytokine IL-23 amplifies IL-17A production by T cells and drives its production by innate cells. We investigated the potential role of IL-23 in small intestinal IRI using mice deficient in IL-23R. IRI was significantly attenuated in *Il23r*^{-/-} mice, as evidenced by a significantly lower injury score (Fig. 5A,B). However, the impairment

of small intestinal IRI in *Il23r*^{-/-} mice was less in *Il17a*^{-/-} mice. To confirm the importance of IL-23 in IRI, WT mice were treated with neutralizing IgG antibody to IL-23, or IgG isotype control, on days -4, -2 and -1 prior to IR. WT mice treated with neutralizing IL-23 antibody developed significantly less SI damage after IR than mice treated with isotype control antibody, as evidenced by a significantly lower injury score (Fig. 5C). These results demonstrate that IL-23 is important for small intestinal IRI.



Key Research Accomplishments

Intracellular complement 3 contributes to ischemic injury.

IL17 and IL23 partially mediate tissue damage in IRI.

Reportable Outcomes

Manuscript submitted to Journal of Immunology- Intracellular activation of complement 3 is responsible for intestinal tissue damage during mesenteric ischemia.

Poster presentation- American Association of Immunologists Annual Meeting: Intracellular activation of complement 3 is responsible for intestinal tissue damage during mesenteric ischemia. New Orleans, LA 2015.

Conclusions

Thus, the above mentioned studies qualifies the goal of our laboratory to ultimately enable the development of novel therapeutic agents and strategies to ameliorate local as well as remote tissue injury in I/R related conditions.

In summary, we have provided evidence that complement is produced by intestinal epithelial cells and that it becomes activated during ischemia by cathepsins. Locally produced complement and its subsequent activation account for the previously unrecognized damage occurring during ischemia. At the clinical level cathepsin inhibition may help with the preservation of organ integrity but it appears that inhibition of circulating complement is necessary to limit damage that occurs after reperfusion.

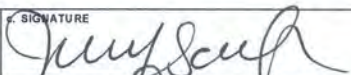
We propose a model for IRI in which ischemia causes the expression of damage-associated neoantigens on intestinal cells. Binding of natural antibodies to these neoantigens results in fixation and activation of complement. Complement binding to receptors, such as C3aR and C5aR, releases innate cytokines, including IL-23, that drive IL-17A production in innate immune cells. IL-17A acts on its receptor on epithelial cells to release chemokines that act in synergy with IL-1 and IL-6 to attract neutrophils. Neutrophils may release endogenous products, which may include IL-17A, that amplify their recruitment. The release of granular contents from neutrophils causes tissue damage, resulting in IRI. Our results suggest that blockade of IL-17A may provide a powerful therapeutic strategy to attenuate IRI.

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REPORT OF INVENTIONS AND SUBCONTRACTS <i>(Pursuant to "Patent Rights" Contract Clause) (See Instructions on back)</i>				Form Approved OMB No. 9000-0095 Expires Aug 31, 2001																																																											
The public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing the burden, to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (9000-0095), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a																																																															
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a. NAME OF AUTHORIZED CONTRACTOR/SUBCONTRACTOR OFFICIAL (Last, First, Middle Initial) Jennifer Sabbagh		b. TITLE Research Administrative Director		c. SIGNATURE 																																																											
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